Supplementary Material for:

Abnormal concentration of porphyrins in serum from COVID-19 patients

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Methods

Patient Recruitment. All participants in the study come from the Basque Country and provided anonymous oral informed consent to clinical investigations using an approved consent form, with evaluation and approval from the corresponding ethics committee (CEIC-E 20-26). The sample size and the study design are summarized in Table 1.

Porphyrin extraction. For porphyrin extraction, 100 μL of 6M HCl were added to 100 μL of the serum sample, incubated at 37 °C for 30 min and centrifugated for 10 min at 10000*g*. The supernatant was filtered (0.22 μm pore size) and centrifuged for 10 min at 4000*g*.

HPLC analysis. Porphyrins were separated by a 60 min gradient elution with a two-component mobile phase consisting of ammonium acetate (1M, pH 5.16, solvent A) and 100% acetonitrile (solvent B). Gradient elution: 0-65% B over 30 min at a flow rate of 1 mL/min. All analyses were performed at 20 °C with fluorescence detection at an excitation wavelength of 405 nm and emission wavelength 610 nm. Extraction was normalized using the riboflavin (RB) signal. A standard commercial lyophilized porphyrin kit was used to calibrate the porphyrins in the chromatogram.

Serum sample preparation for ^{1}H NMR measurements. Each sample of 500 μL serum was defrozen and then prepared for NMR measurements. The phosphate buffer contained trimethylsilylpropionic acid-d^{4} sodium salt (TSP) and 10% D_2O.

^{1}H NMR spectroscopy. All ^{1}H-NMR spectra were measured on a 600 MHz IVDr spectrometer at 310 K as described previously (1). Experiments recorded: a quantitative one-dimensional (1D) ^{1}H spectrum with water presaturation, a 1D ^{1}H CPMG experiment with suppression of protein background and a 2D J-resolved spectrum. A list of metabolites and lipoprotein subclasses was then obtained using the B.I.-LISA identification software (Bruker BioSpin), which also calculates absolute concentrations in mmol/L.

Statistical analysis of metabolomics data. Coefficients and p-values associated to COVID-19 variable were extracted from models. For each value, 95% confidence interval was also calculated.

(1) Bruzzone C, Loizaga-Iriarte A, Sanchez-Mosquera P, Gil-Redondo R, Astobiza I, Diercks T, et al. 1H-NMR-based urine metabolomics reveals signs of enhanced carbon and nitrogen recycling in prostate cancer. *J Proteome Res*. 2020;19(6):2419–28.